SHORTENING OF MICROVILLI DURING THE MATURATION OF STARFISH OOCYTE FROM WHICH VITELLINE COAT WAS REMOVED

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SHORTENING OF MICROVILLI DURING THE MATURATION OF STARFISH OOCYTE FROM WHICH VITELLINE COAT WAS REMOVED[1)]

Setsuro Hirai* and Hisato Shida[2)]**

** Marine Biological Station, Tôhoku University, Asamushi, Aomori, 039-34 Japan and ** Department of Anatomy, Tôhoku University School of Medicine, Sendai 980, Japan*

The oocytes of the starfish, *Asterina pectinifera*, were examined with scanning electron microscope to observe the morphological changes of the microvilli during oocyte maturation. The round tips of the microvilli protruded on the vitelline coat in the immature oocyte placed in calcium-free sea water, in which the follicular cells dispersed. The numerous slender microvilli were observed on the surface of the denuded immature oocyte from which the vitelline coat was mechanically removed after being elevated with ionophore A–23187. When the vitelline coat-free oocytes were exposed to 1-methyladenine, the germinal vesicle broken down normally and the microvilli transformed from slender to short. The shortening of the microvilli, which occurs independently on the changes of the vitelline coat after 1-methyladenine application, seems to be one of the maturation events at the oocyte surface.

In starfish, oocyte maturation is induced by 1-methyladenine (Kanatani *et al.* 1969). Kanatani and Hiramoto (1970) have demonstrated that the site of action of 1-methyladenine in inducing oocyte maturation seems to be egg surface. Recently we succeeded in complete removal of the vitelline coat from immature oocytes, from which the vitelline coat was elevated by exposing them to divalent ionophore A–23187 following the immersion with calcium-free sea water. These vitelline coat-free immature oocytes underwent breakdown of germinal vesicles after the treatment with 1-methyladenine sea water (Shida and Hirai 1978).

On the other hand, we have demonstrated by the transmission electron microscope that the vitelline coat became flat and thin after the treatment with 1-methyladenine. The microvilli of the oocyte surface protruding into the vitelline coat became fewer in number (Hirai *et al.* 1971). But it was unknown whether the microvilli are withdrawn or detached from the oocyte surface, because the presence of the vitelline coat on the oocyte surface prevented to observe entirely the changes of the microvilli.

In this study, we observed the morphological changes of the microvilli on the surface of the vitelline coat-free oocyte during the oocyte maturation.

1) Contribution from the Marine Biological Station, Tôhoku University, No. 433

2) 平井 説郎, 志田 寿人

MATERIAL AND METHODS

Gamates The starfish, *Asterina pectinifera*, were collected at Asamushi and kept in the labolatory aquarium supplied with circulating cold sea water at the Marine Biological Station of Tohoku University, Asamushi, Aomori. Fullgrown oocytes were isolated from ovary fragments with the forceps into calcium-free sea water. The oocytes do not undergo spontaneous maturation when isolated into sea water.

Reagents Artificial sea water used in this study was Herbst's calcium-free sea water (CFSW, PH 8.2). 1-Methyladenine (1-MA, Sigma Chemical Co.) was dissolved in deionized water at a concentration of 10^{-3}M and diluted with CFSW to 10^{-6}M before use. Divalent ionophore A–23187 was obtained from R, L. Hamill, Eli Lilly Co., Indianapolis, Ind. and dissolved in demethylsulfoxide at a concentration of 4 mM as a stock solution. In the experiments 10 μl of 4 mM ionophore A–23187 was added to 1 ml oocyte-CFSW suspension.

Preparation for scanning electron microscope (SEM) Examined oocytes were fixed for a minimum of 2 hrs in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (PH 7.8) containing 8.6% sucrose, followed by dehydration in a graded series of ethanol. The ethanol was replaced by amylacetate, and the material dried at critical point in carbon dioxide with a critical point dryer (Hitachi, HCP–1). The samples were coated with gold and viewed with SEM (Hitachi-Akashi, MSM–4).

Removal of vitelline coat The method for removing the vitelline coat from immature oocytes was described in detail in our previous paper (Shida and Hirai 1978). The immature oocytes with germinal vesicles were immersed in CFSW for 40 min and then treated with divalent ionophore A–23187 for 15 min. Consequently, the vitelline coat was elevated highly in CFSW containig ionophore A–23187. These immature oocytes with the elevated vitelline coats were passed through the mesh with the microsyringe. These elevated vitelline coats were broken by the mesh and the vitelline coat-free oocytes were obtained.

RESULTS AND DISCUSSION

When the immature oocytes were placed in CFSW, follicular layer dispersed from the surface of the oocytes, and the germinal vesicle was kept intact. At the surface of these oocytes, it was observed by SEM that the tips of the microvilli protruded outside the vitelline coat (Fig. 1A). The diameter of these tips is about 0.24 μm. After the induction of the breakdown of the germinal vesicle (GVBD) with 1-MA sea water, these tips of the microvilli were not observed on the surface of the vitelline coat which became rough (Fig. 1B).

Recently, it was reported that in *Pisaster giganteus* the vitelline coat changes from a coarse to a ruffled surface with the treatment of 1-MA (Rosenberg *et al* 1977), and in *Patiria miniata* the microvilli appear to be more numerous and prominent in the matured oocyte (Lee *et al* 1977). The changes on the surface of the oocyte

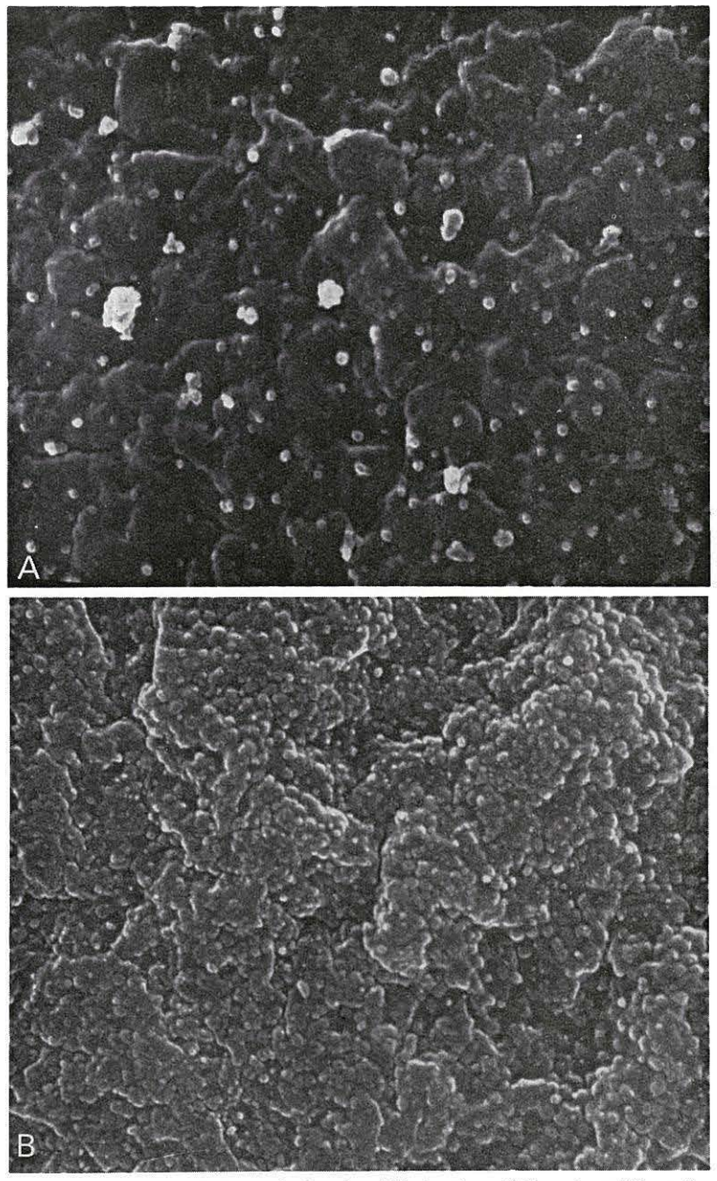

Fig. 1. Scanning electron micrograph showing (A) the tips of the microvilli on the vitelline coat of the immature oocyte after the treatment with CFSW for 40 min (×7000) and (B) the surface of the vitelline coat after GVBD (×7000). The oocyte was fixed at 45 min after 1-MA application.

sometimes may take a different form in different species. But we observed previously with transmission electron microscope that the microvilli protruding into the vitelline coat at the surface of the immature oocytes were fewer in number (HIRAI *et al.* 1971). These our findings seems to suggest that the microvilli are withdrawn from the surface of the vitelline coat during the oocyte maturation. Therefore, the change of the microvilli in the vitelline coat-free oocyte was observed before and after GVBD. When immature oocytes were exposed to ionophore A–23187 in CFSW after the treatment with CFSW for 40 min, the vitelline coat elevated completely and separated from the whole oocyte surface, but GVBD did not occur. These elevated vitelline coats were completely removed from the immature oocytes by passing through the mesh. These vitelline coat-free immature oocytes almost did not show any changes and the germinal vesicles was kept intact for 3 hrs in normal sea water after this procedure. Many slender microvilli (length; about 1.3 μm, width; about 0.22 μm) were observed on the surface of the vitelline coat-free oocyte (Fig. 2B, 3A). Fig. 2B shows that the vitelline coats was completely removed from the whole oocyte surface. When these vitelline coat-free oocytes were treated with 1-MA sea water (10^{-6}M), GVBD were induced normally (SHIDA and HIRAI 1978). On the surface of the vitelline coat-free mature oocyte the microvilli became very short (length; about 0.29 μm) and appeared as dumpy papillae (Fig. 3B). The slender microvilli may shorten during the oocyte maturation.

It was seen that lack of Ca^{2+} in the sea water may have no effect on the morphological changes at the oocyte surface. CAYER *et al* (1975) reported that the treatment with calcium-free sea water did not cause any changes on the surface in starfish oocytes. The oocytes that treated with ionophore A–23187 underwent the breakdown of the cortical granule (SCHUETZ 1975). The microvilli protruded over the thick vitelline coat surrounding the immature oocyte before the treatment of ionophore A–23187. Their tips were observed at the surface of the vitelline coat (Fig. 1A). Many slender microvilli observed on the surface of the vitelline coat-free immature oocytes, in which cortical granules were broken down by ionophore A–23187. The tips of these many slender microvilli are considered protruding over vitelline coat before the treatment of ionophore A–23187.

From these results it is considered that the slender microvilli protruded into the vitelline coat and most of them run through the vitelline coat in the immature oocyte, and they shorten and withdrew into the vitelline coat during oocyte maturation after exposing to 1-MA.

The shortening of the microvilli of the immature oocytes, from which the vitelline coats were removed, occured after the application of 1-MA without any participation of the vitelline coat. This shortening of microvilli seems to be one of the maturation events induced by 1-MA at the oocyte surface.

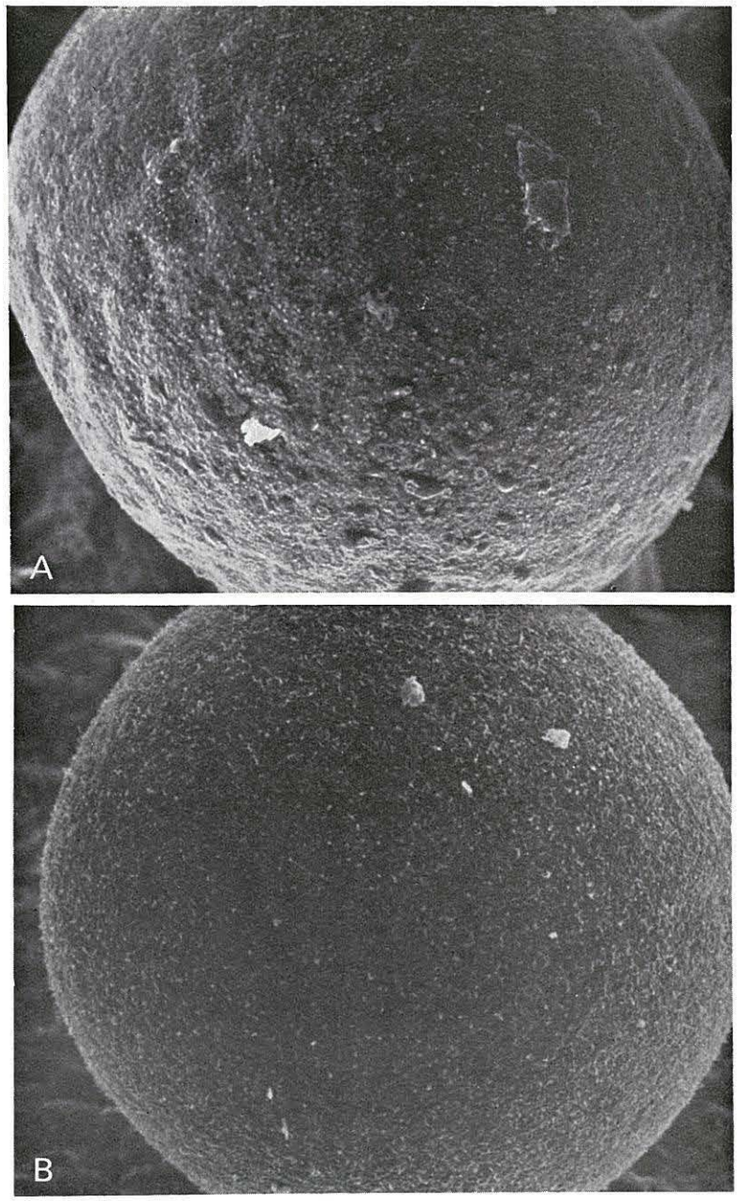

Fig. 2. Scanning electron micrograph of the immature oocyte with the vitelline coat that treated CFSW alone (A) (×1000) and the vitelline coat-free immature oocyte (B) (×1000).

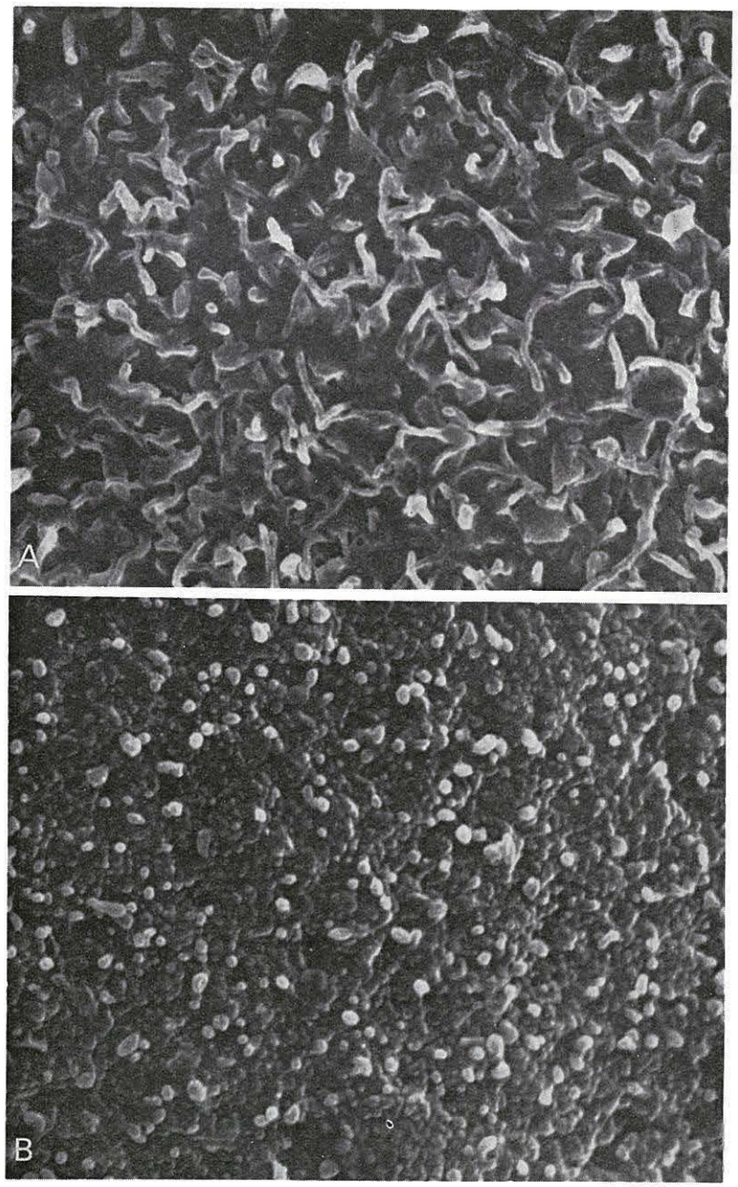

Fig. 3. Scanning electron micrograph showing (A) many slender microvilli on the surface of the vitelline coat-free immature oocyte (×7000) and (B) shortened microvilli of the vitelline coat-free matured oocyte (×7000). The oocyte was fixed at 45 min after 1-MA application.

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